Plant Root Exudates and Their Influence upon Soil Microorganisms

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One of the major factors determining the behaviour of plant pathogens in soil is the availability of nutrients, and it is in this respect that the exudation of organic compounds from plant roots plays an important role in the problem of biological control of root diseases. From the time Hiltner (1904) observed that microorganisms were more abundant in soil near plant roots than in distant soil there has been considerable speculation as to whether soluble organic substances were wholly or partly responsible for this stimulation of the microflora around plant roots.

The two aspects of root-exudate research not dealt with in this review are the interactions between higher plants in relation to root exudates (reviewed by Börner, 1960; Wood, 1960) and the release of nitrogen from nodulated legumes (Wyss and Wilson, 1941; Virtanen, von Hansen, and Laine, 1937; Butler and Bathurst, 1956). This review will deal with the nature of plant root exudates with emphasis on their influence on both the saprophytic and pathogenic soil microflora.

In the field of root-exudate research there are four papers of historic interest reporting the release of nitrogen compounds, sugars, phosphatides and nematode-stimulating factors from roots. The first conclusive evidence of exudation from roots was provided by Knudson (1920), who showed that peas and maize grown under aseptic conditions in sucrose solution produced considerable quantities of reducing sugars. Knudson proposed that the sucrose was absorbed by roots and converted to reducing sugars, which were excreted. Lyon and Wilson (1921) found that organic nitrogen was released from maize roots growing under sterile conditions in large-capacity vessels; they concluded that this material was released by living roots rather than the sloughed-off root cells which accumulated at the bottom of the vessels. Phosphatides were reported by Cranner (1922) as coming from the roots of seedlings and mature plants. The first conclusive evidence of the stimulation of specific organisms by root exudates was that of O'Brien and Prentice (1930). By showing that the cysts of the potato eelworm (*Heterodera schachtii,* now *H. rostochiensis* Woll.) hatched in the presence of root washings of potato but not the washings of beet, rape, lupin, mustard, or oat roots, they demonstrated the biological specificity of the exudates of different plants.

After these early reports little was done in the field until this last decade, during which there has been considerable interest and research into the nature and effects of root exudates. This delay in following up the earlier discoveries was probably due to two major technical problems, namely the growth of plants in sufficient numbers under sterile conditions, and the identification of organic materials at the low concentrations at which they occur in root exudates.

ASEPTIC CULTURE OF PLANTS.—As it is not possible to determine the nature of true root exudates unless plants are grown in the absence of microorganisms, one is faced with the problems of seed sterilization and growth of the plants under aseptic conditions until the exudate is removed for analysis. Seeds can be surface-sterilized without difficulty with mercuric chloride, hypochlorite, and other compounds and germinated on agar (as a sterility check and to remove initial seed exudates), before planting in a suitable apparatus for aseptic culture. Certain plant species, e.g. grasses and cereals, often contain an internal microflora that makes it impossible to conduct exudation studies under the ideal conditions of complete asepsis. In such cases the soaking and heat treatment of seeds may destroy some of these contaminants. If this is not successful, then the presence of contaminants should be noted in any consideration of the nature of the root exudates.

Many methods have been proposed for the aseptic culture of plants. The apparatus used depends to a large extent upon the number of plants required and the age to which the plants will be grown. Several effective types of apparatus have been developed for growing plants with sterile roots. In some of these the tops remain in the open (Nilsson, 1957; Waris, 1958; Szember, 1959; Reuszer, 1949, 1962; Stotzky, Culbreth, and Mish, 1962; Bradfute et al., 1962). The major problems with these methods are the risk of contamination and the fact that it is difficult to grow the large number of plants generally required for root-exudate studies.

The simplest and most effective method of overcoming these problems is to enclose the entire plant. This can be done simply and effectively in large test tubes. The plants are supported by a stainless-steel mesh with the roots in nutrient solution, which can be changed and sampled for analysis when necessary. With seedlings growing under such conditions aeration does not appear to be necessary for healthy root and root-hair development. This technique has the disadvantages that the plants grow in a high-humidity environment and the studies are confined to seedlings.

There is also the possibility of contamination of the root exudate with seed and leaf exudates. Studies on the effects of environment and age of plant on exudation indicate, however, that such contamination is of minor importance.

METHODS OF ANALYSIS.—Although the quantities of organic material exuded from roots are sufficient to support large populations of microorganisms, they are often too low to be determined without the use of sensitive techniques. The advent of paper and column chromatography and biological assay, however, has made it possible to analyse root exudates for many organic compounds, such as sugars, amino acids, organic acids, and nucleic acid derivatives, which might be expected in root exudate.

Microbiological assay methods, developed for the detection of minute quantities of vitamins, can be applied equally successfully to the analysis of root exudates and the detection of biologically significant quantities of growth factors.

Direct observations of exudation from sterile roots, as carried out by Pearson and Parkinson (1961) and by Schroth and Snyder (1961, 1962), have proved useful in determining the sites of major exudation on roots. The exudates from sterile roots can be absorbed onto filter paper and, by using selective sprays, a range of compounds coming from different parts of the root system may be detected. As a further refinement of the technique which may yield more data, these areas may be eluted with sprays prior to development, and the subsequent eluate chromatographed.

Bioassay techniques based on the growth responses of specific organisms (generally plant pathogens) to exudates have often been reported, e.g. spore-germination factors, fungus-attracting factors; but these give little or no information on the composition of the exudates. The precise identification of the compounds involved is often long and tedious and necessitates the services of skilled organic chemists. This has been the case with the potato-eelworm-hatching factor first reported by O'Brien and Prentice (1930) and subsequently partially characterized by a team of organic chemists headed by Todd (Calum, Raistrick, and Todd, 1949; Calum, Todd, and Waring, 1949; Marrian et al., 1949; Russell, Todd, and Waring, 1949).

The use of *Neurospora* mutants with different nutrient requirements to determine whether different compounds in the exudate originated from different sites along the root has been developed by Frenzel (1960). In this technique direct microscopy is combined with the use of specific *Neurospora* mutants.

In the root-exudate studies little use has been made of radioactive tracer techniques. Their application would help elucidate the mechanisms involved and enable the detection of fluctuations in exudation with changes of environment.

NATURE OF EXUDATES.—*Carbohydrates.*—At least ten sugars, including an oligosaccharide, have been identified in the exudates of a wide range of plants. Although few quantitative estimates of the sugars have been made, glucose and fructose are generally the most abundant. The roots of oats, grown aseptically in nutrient solution, sand, or glass beads, have been found to be covered with a brown mucilaginous material (Rovira, unpublished). This material is insoluble in cold water and soluble in hot water, and is a short-chain oligosaccharide releasing glucose and fructose upon hydrolysis. Under nonsterile conditions this mucilage becomes densely colonized by microorganisms and obviously forms a readily available substrate. Jenny and Grossenbacher (1963) have reported a "mucigel" on the roots of barley seedlings, but this appears to be a pectic acid polymer and is not densely colonized by microorganisms. Dart and Mercer (1964) have also demonstrated the presence of an external gelatinous material on the roots of barrel medic (*Medicago tribuloides*) grown under aseptic conditions (Fig. 1). The nature of this mucilage on medic roots has not yet been characterized.

The range of sugars reported in exudates and the plants from which they were released (Table 1) indicates that exudation of sugars is a general phenomenon playing an important role in the nonspecific stimulation of microorganisms in the rhizosphere.

Amino acids.—Amino acids form the most studied group of compounds in exudates from plant roots. To date 23 have been reported in the exudates of 15 dif-

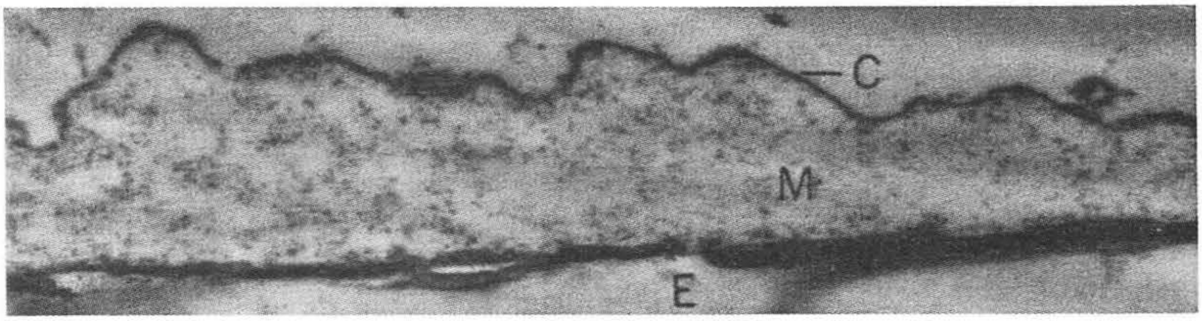


Fig. 1. Mucilaginous material on a sterile root of *Medicago tribuloides* 5 to 15 mm from the tip. (× 20,000) E = epidermal cell; M = mucilaginous layer; C = "cuticle." (P. J. Dart and F. V. Mercer. Arch. Mikrobiol. 47: 344-378. 1964.)

TABLE 1. Carbohydrates detected in root exudate

Compound	Plant	Reference
Glucose	Peas, wheat	Lundegårdh and Stenlid, 1944
	Peas	Rovira, 1956*a*
	Soybean, barley, wheat	Katznelson, Rouatt, and Payne, 1955
	Wheat	Vrany, Vancura, and Macura, 1962
	Oats, mustard	Bhuvaneswari and Subba-Rao, 1957
	Oats	Rivière, 1960; Rovira, 1956*a*
	White pine	Slankis, 1958
Fructose	Peas, oats	Rovira, 1956*a*
	Sorghum, mustard	Bhuvaneswari and Subba-Rao, 1957
Sucrose	Bean	Schroth and Snyder, 1961
Xylose	Sorghum, mustard	Bhuvaneswari and Subba-Rao, 1957
	Wheat	Vrany, Vancura, and Macura, 1962
Maltose	Mustard	Bhuvaneswari and Subba-Rao, 1957
	Bean	Schroth and Snyder, 1961
Rhamnose	Wheat	Vrany, Vancura, and Macura, 1962
Arabinose Xylose Raffinose	White pine	Slankis, 1958
	Wheat (traces)	Rivière, 1960
Oligosaccharide	Oats	Rovira (unpublished)
	Wheat	Vrany, Vancura, and Macura, 1962
Unidentified	Sugar beet	Geller, 1954

ferent plant species (Table 2). The spectra of amino acids reported for the different plants vary considerably; but such comparisons between plants may be misleading because of the different conditions under which the experiments were conducted by different investigators. Even the exudation patterns for a single plant species such as wheat as found by four different workers vary considerably (Table 2, columns 11, 12, 13, 14). In a direct comparison between the exudates of different plants grown under identical conditions, marked differences were apparent in the amounts and types of amino acids coming from roots (Rovira, 1956*a*, 1959). A list of the principal amino acids exuded from peas and oats during their first 10 days of growth in sterile quartz sand is given in Table 3. It required seven times as much exudate from oats as from peas to yield a suitable chromatogram; this indicates the quantitative differences between the plants. A comparison of the amino acids in pea- and oat-root exudate showed homoserine, threonine, α-alanine, glutamine, asparagine, and serine to be dominant in pea-root exudate; while in oat-root exudate serine, lysine, and glycine were the most abundant. These qualitative and quantitative differences in the amino acid patterns of exudates from different plant species grown under identical conditions will, no doubt, influence the balance of organisms developing in the rhizosphere, and, even without the more complex compounds, may perhaps determine the susceptibility and resistance of plants to root pathogens.

The influence of triiodobenzoic acid and chloramphenicol as foliar sprays on the exudation of amino acids has been studied by Vrany, Vancura, and Macura (1962). They found that chloramphenicol caused a general increase in amino acids in the exudate as well as a relative increase in α- and β-alanine and a decrease in phenylalanine.

Vitamins.—Ten vitamins have been identified in exudates from a wide variety of plants (Table 4) and, although the levels are generally low, these may be sufficient to meet the requirements of some of the vitamin-requiring microorganisms of the rhizosphere.

In an analysis for six vitamins in the exudates from lucerne, field pea, tomato, phalaris, and six clover species, biotin was found to be consistently present at low but biologically active levels (Rovira and Harris, 1961). Pantothenate and niacin generally occurred in the exudate, thiamin was found only occasionally, riboflavine infrequently, and pyridoxine never. The sporadic occurrence of thiamin in legume-root exudate was interesting in view of the statement by Wilson (1940) that "Thiamin can be classed as an essential growth factor for the rhizobia." The exudate findings indicate that the rhizobia must depend upon the synthesis of thiamin by neighbouring organisms rather than upon the host plant. Similarly, it is likely that the high proportion of vitamin-requiring bacteria which occur in the rhizosphere (Wallace and Lochhead, 1949) depends more on vitamin-synthesizing microorganisms than root exudates for growth.

Organic acids.—Although organic acids are involved in the uptake of nutrients by roots and are found inside the root at significant levels, there are few reports of these materials occurring in root exudate (Table 5). The only quantitative data are those of Rivière (1959, 1960), who found that a single wheat plant grown aseptically in nutrient solution to the tillering stage (6 weeks) exuded 13 mg acetic, 3.5 mg propionic, 2 mg butyric, and 1.5 mg valeric acids, which would be significant in the nutrition of the rhizosphere population.

Considering the part played by organic acids in root metabolism and probable fluctuations in levels accord-

TABLE 2. Amino acids detected in root exudate

Amino acid	Sunflower[1]	Oats[2]	Maize[3]	Peas[4]	Peas[5]	Rice[6]	White clover[7]	Perennial rye grass[8]	Wormwood[9]	Tomato[10]	Wheat[11]	Wheat[12]	Wheat[13]	Wheat[14]	Subterranean clover[15]	Phalaris[16]	Monterey Pine[17]	Flax[18]	Cotton[19]
Leucine/isoleucine	+	+	+	+	+	—	+	+	+	—	+	—	+	+	+	+	+	—	+
Valine	+	+	+	+	—	—	+	+	+	—	—	+	+	+	+	+	+	—	—
γ-Aminobutyric acid	Tr*	+	—	+	—	—	—	—	—	—	—	+	+	+	+	+	+	+	—
Glutamine	+	+	+	+	+	—	—	—	—	—	+	+	+	+	+	+	+	—	—
α-Alanine	+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	—
Asparagine	+	+	+	+	+	+	—	—	—	—	+	+	+	+	+	+	+	—	—
Serine	Tr	+	+	+	—	—	Tr	Tr	+	—	—	+	+	+	+	+	+	—	+
Glutamic acid	+	+	+	+	+	+	+	+	Tr	+	—	+	+	+	+	+	+	+	—
Aspartic acid	+	+	+	+	+	+	+	+	+	+	—	—	+	+	+	+	+	+	+
Cystine/cysteine	—	+	—	—	+	+	—	—	—	+	—	+	—	+	+	+	—	—	+
Glycine	—	+	—	—	+	—	—	—	—	+	—	+	+	+	+	+	+	—	+
Phenylalanine	+	+	—	+	—	—	—	—	—	—	—	—	—	+	+	—	Tr	—	+
Threonine	+	+	—	+	+	—	—	—	—	—	—	+	—	+	+	+	+	—	+
Tyrosine	—	+	—	+	—	+	—	—	—	+	—	—	+	—	—	—	—	—	+
Lysine	—	+	—	+	—	+	—	—	—	—	+	—	—	—	—	—	—	—	+
Proline	—	—	—	—	+	—	—	—	—	—	—	+	+	—	—	—	+	—	+
Methionine	—	—	—	+	—	+	—	—	—	—	—	—	+	+	+	+	+	—	—
Tryptophane	—	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	+	—	—
Homoserine	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
β-Alanine	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	+	—	—
Arginine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+

* Tr = trace; — = absent or not recorded.

References: [1] Frenzel, 1957; [2] Katznelson, Rouatt, and Payne, 1955; Parkinson, 1955; Rovira, 1956*a*; Martin, 1957; [3] Kandler, 1951; Frank, 1954; [4] Katznelson, Rouatt, and Payne, 1955, Rovira, 1956*a*; [5] Buxton, 1962; [6] Frank, 1954; [7] Andal, Bhuvaneswari, and Subba-Rao, 1956; [8] Linskens and Knapp, 1955; [9] Linskens and Knapp, 1955; [10] Kalyanasundarum, 1958; Rovira, 1959; [11] Katznelson, Rouatt, and Payne, 1955; [12] Rivière, 1960; [13] Tèsar and Kutacek, 1955; [14] Vrany, Vancura, and Macura, 1962; [15] Rovira, 1959; [16] Rovira, 1959; [17] Bowen, 1963; [18] Börner, Martin, and Clauss, 1959; [19] Sulochana, 1962*a*.

ing to the state of aeration and metabolism of the root, it is not unexpected to find them in the exudate. This group of compounds deserves much more study, not only because they form a source of readily available substrates, but also because of secondary effects, such as the modification of pH in the rhizosphere and the chelation of metals.

Nucleotides, flavonones, and enzymes.—In a study with excised roots of wheat and peas, Lundegårdh and

TABLE 3. Amino acid exudation patterns of peas and oats after 21 days

	Peas (3)*	Oats (20)*
Asparagine	+++	++
Aspartic acid	++	+
Serine	+++	+++
Glutamine	++++	+
Homoserine	++++	—
α-Alanine	++++	+
Leucine	+	++
Lysine	++	+++
Threonine	++++	+/—
Glycine	—	+++

* Number of plants required to provide material for a single chromatogram.

TABLE 4. Growth factors present in root exudate

Compound	Plant	Reference
Biotin Thiamin	Flax Rice, bean, clover	West, 1939 Bhuvaneswari and Sulochana, 1955
"M" factor	Pine, peas, tomato	Melin and Rama Das, 1954
Biotin Thiamin	Maize, peas	Meshkov, 1959
Biotin Pantothenate Niacin	Clover species (six), lucerne, phalaris, tomato	Rovira and Harris, 1961
Biotin Thiamin Choline Inositol Pyridoxine p-Amino benzoic acid	Cotton	Sulochana, 1962*b*
n-Methyl nicotinic acid	Radish	Dodman, Kerr, and Atkinson*

* R. Dodman, A. Kerr, and M. R. Atkinson, personal communication.

TABLE 5. Organic acids in root exudate

Compound	Plant	Reference
Tartaric Oxalic	Sorghum, mustard	Bhuvaneswari and Subba-Rao, 1957
	Wheat	Vrany, Vancura, and Macura, 1962
Citric	Mustard	Bhuvaneswari and Subba-Rao, 1957
Malic	Mustard	Bhuvaneswari and Subba-Rao, 1957
	Wheat	Rivière, 1959, 1960
Acetic Propionic Butyric Valeric	Wheat	Rivière, 1959, 1960
Citric Succinic Fumaric Glycolic traces	Wheat	Rivière, 1959, 1960 Vrany, Vancura, and Macura, 1962

Stenlid (1944) demonstrated that 3,4 dioxyflavonone, adenine, and guanine were released into the surrounding medium (Table 6). The exudation of these compounds was linked with root respiration, and the authors proposed that leakage took place from the zone of active cell division immediately behind the root cap. It is in this zone that nucleic acids are being synthesized and respiration involving 3,4 dioxyflavonone is greatest. Also reported in Table 6 are the findings of Fries and Forsman (1951) of nucleic acid derivatives coming from roots.

The presence of exoenzymes associated with roots was demonstrated by Rogers, Pearson, and Pierre (1940, 1942). They found that intense phosphatase activity was associated with the gelatinous material surrounding the roots of maize. Although this material contained free nuclei and empty cell walls of sloughed-off root-cap material, they considered that the phosphatases and gel were exuded directly from the root before disintegration of the cell debris. Krasil'nikov (1952) found that the exudates from wheat, maize, and peas contained invertase, amylase, and protease; and Krasil'nikov and Kotelov (1959) also reported phosphatase in the external environment of the roots.

The work of Knudson (1920) reported earlier in this review indicates the caution required in the interpretation of results. His initial conclusions from the observation that glucose and fructose appeared in a sucrose solution in which plants were growing under sterile conditions was that invertase was being exuded. However, he demonstrated that the sucrose was first absorbed by the plant, hydrolysed to the constituent sugars inside the plant, and these subsequently exuded.

The polygalacturonase reported by Ljunggren and Fåhraeus (1959, 1961) to be released into distilled water by the roots of lucerne and clover occurred as a result of the stimulus of infective rhizobia or polysaccharide extracts of these organisms. This reaction was so specific that no polygalacturonase was produced when noninfective rhizobia colonized the root and demonstrates the effects which different microorganisms have on the composition of exudates.

Miscellaneous compounds.—A wide range of miscellaneous compounds is released from roots, and several of these are toxic to microorganisms (Table 7), e.g. hydrocyanic acid, glycosides, and saponins. This demonstrates that in any study of the effects of root exudates on the soil microflora, consideration must be made of the inhibitory as well as the stimulatory materials.

Elkan (1961) showed that plants with only a single gene difference can vary markedly in their effects upon the rhizosphere population. He found that exudate from nonnodulating strains of soybean altered the morphology of *Rhizobium* in culture solutions and would also prevent nodulation of the normally nodulating strains of soybean.

Factors affecting nematodes.—There is considerable evidence of the ability of roots to attract nematodes in both soil and culture media (Table 8), although the actual materials have seldom been identified. Bird (1959) found that the larvae of *Meloidogyne javanica* and *M. hapla* were attracted by reducing substances and postulated that attraction to the rapidly elongating area of the root was due to the lower oxidation-reduction potential in this area, but later (Bird, 1960) he considered that root exudates could also be significant.

The work of Wallace (1958) on the emergence of eelworm cysts and the subsequent migration of larvae showed conclusively that exudate influenced a considerable zone of sand surrounding the roots. When sand was held at 15 cm suction, the cyst germination factor(s) diffused 3.0 cm from the root and larvae were attracted towards the root from distances of up to

TABLE 6. Nucleotides, flavonones and enzymes detected in root exudate

Compound	Plant	Reference
Flavonone	Peas, wheat	Lundegårdh and Stenlid, 1944
Adenine	Peas, wheat	Lundegårdh and Stenlid, 1944
Guanine	Peas, wheat	Lundegårdh and Stenlid, 1944
Uridine + cytidine	Peas	Fries and Forsman, 1951
U.V.-absorbing compounds	Peas, oats	Rovira, 1956*a*
Phosphatase	Maize	Rogers, Pearson and Pierre, 1942 Krasil'nikov and Kotelov, 1959
Invertase Amylase Protease	Wheat, maize, peas	Krasil'nikov, 1952
Polygalacturonase	Lucerne, clover	Ljunggren and Fåhraeus, 1959, 1961

TABLE 7. Miscellaneous compounds reported in root exudate

Compound	Plant	Reference
Auxins	Pinto bean	Mitchell, Lindner, and Robinson, 1961
Scopoletin (6 methoxy-7-hydroxycoumarin)	Oats	Eberhardt, 1954 Martin, 1958
Fluorescent substances	Oats	Eberhardt, 1955
	Peas	Rovira, 1956*a*
	Lucerne	Mishustin and Naumova, 1955
	Banana	Buxton, 1962
Hydrocyanic acid	Flax	Timonin, 1941
Glycosides	Asparagus	Rohde and Jenkins, 1958
	Oats	Schönbeck, 1958
Saponins (glucosides)	Lucerne	Mishustin and Naumova, 1955
Organic phosphorus compounds	Maize Pumpkin	Popatov and Molnarne Kerestes, 1956
γ-MnO_2-dissolving compound	Oats, vetch	Bromfield, 1959
Bentonite colouring compound	Red clover	Nutman, 1951
Nodulation inhibitor	Soybean (nonnodulating strain)	Elkan, 1961
Azotobacter growth stimulator	Barley, wheat	Vancura and Macura, 1961

4.5 cm. Diffusion distances were reduced when the water content of the sand was reduced. There are few data on the distance of diffusion of exudates from roots in soil, but this also will be influenced by water content. The adsorption of cationic materials in the exudate by the clay fraction will restrict their effects upon the microflora to a small zone around the root, but neutral or anionic nutrients should diffuse considerable distances. The actual distance involved will also be affected by the rate of decomposition of these compounds during their diffusion through soil.

The specificity of some of these factors was shown by O'Brien and Prentice (1930), who found that potato root exudates stimulated the hatching of potato eelworm cysts, whereas the exudates from beet, rape, lupin, mustard, and oats had no effect. Subsequent work by Calum, Raistrick, and Todd (1949) showed that other members of the Solanaceae also liberated the factor. A large-scale research programme to assess the possibility of controlling potato eelworm with this exudate or a chemical analogue involved growing 150,000 tomato plants in sand culture and leaching on alternate days to obtain 12 g of crude factor per month. The active fraction has been shown to contain a lactone ring, is acidic in nature, and has been named "eclipic acid." Ellanby (1958*b*) and Ellanby and Gilbert (1960) have shown that this factor has certain affinities with cardiac glycosides, probably owing to an unsaturated lactone ring; but precise characterization has not yet been achieved. This factor has been studied by several workers (see Table 8) and considerable information has been obtained on the conditions required for maximum production and its stability to heat and oxidation. Fenwick (1956) found that the activity of the potato-root diffusate was almost completely destroyed 4 days after addition to soil.

Factors affecting fungi.—The many reports of fungi in soil being affected by roots or root exudates are summarized in Table 9. The specificity of root exudate in stimulating plant pathogens was demonstrated by Kerr (1956), using the cellophane-bag technique. Kerr grew sterile seedlings inside cellophane bags buried in soil inoculated with *Pellicularia filamentosa* and found an intense development of the pathogen on the cellophane opposite the roots of the two susceptible hosts, lettuce and radish, but no stimulation opposite tomato roots, which are not susceptible.

Buxton (1957) also demonstrated a definite specificity with relation to the germination of spores of *Fusarium oxysporum* Fr. f. *pisi* (Linf.) Snyd. and Hans. in the exudates of three pea varieties differing in susceptibility to this pathogen. Exudate from a wilt-resistant variety inhibited spore germination, whereas exudate from a susceptible plant stimulated spore germination. But the exudates showed no differences in their effects on the rate of growth of germ tubes from germinated spores or of the developing mycelium; specificity was restricted to spore germination.

On the other hand, Schroth and Hendrix (1962) found that chlamydospores of *Fusarium solani* f. *phaseoli* germinated in the presence of exudates from many nonsusceptible plants as well as the host plant.

The influence a host plant can have on a pathogenic fungus was demonstrated by Buxton (1958). He found that after several subcultures of *Fusarium oxysporum* f. *pisi* in a medium containing root exudate of a wilt-resistant pea variety, the fungus increased in its pathogenicity towards this wilt-resistant variety. Buxton suggests that this change in pathogenicity may be due to mutagenic substances in the exudate increasing the mutation rate of the fungus or an adaptation by the fungus to either inhibitory materials or previously unavailable nutrients in the root exudate of resistant varieties, but he offers no evidence in support of either hypothesis.

FACTORS AFFECTING EXUDATION.—One of the problems in assessing the significance of analyses of root exudates is that exudation is affected by many factors. Katznelson, Rouatt, and Payne (1954, 1955) demonstrated that temporary wilting of plants increased the

TABLE 8. Nematode-stimulating factors from root exudate

Compound	Plant	Reference
Potato eelworm cyst hatching factor (*Heterodera rostochiensis* Woll.)	Potato and other *Solanaceae*	O'Brien and Prentice, 1930 Calum, Raistrick, and Todd, 1949 Calum, Todd, and Waring, 1949 Marrian et al., 1949 Russell, Todd, and Waring, 1949 Fenwick, 1952, 1956, 1957 Carroll et al., 1958 Widdowson, 1958*a,b,c* Widdowson and Wiltshire, 1958 Hague, 1958 Ellenby, 1958*a,b* Ellenby and Gilbert, 1960 Hartwell, Dahlstrom, and Neal, 1960
Beet eelworm cyst hatching factor (*H. schachtii* Schmidt)	Beet	Winslow and Ludwig, 1957 Wallace, 1957 Carroll, 1958
	Rape	Winner, 1958
Cabbage eelworm cyst hatching factor (*H. cruciferae*)	Black mustard	Carroll, 1958
Hop eelworm cyst hatching factor (*H. humili*)	Hop	Carroll, 1958
Heterodera spp. attractors	Potato	Widdowson, Doncaster, and Fenwick, 1958
	Cress	Wallace, 1958
	Tomato	Weischer, 1959 Jones, 1960
Meloidogyne spp. attractors	Tomato	Bird, 1959, 1960 Peacock, 1961

TABLE 9. Factors in root exudates affecting fungi

Factor	Plant	Fungus affected	Reference
Mycelium-growth stimulators	Radish Lettuce	*Pellicularia filamentosa*	1
	Strawberry	*Rhizoctonia* sp.	2
Mycelium-growth inhibitors	Oats	*Byssochlamys nivea*	3
Spore-germination stimulator	Potato *Datura* spp.	*Spongospora subterranea*	4
	Tomato	*Colletotrichum atramentarium*	5
	Turnip	*Pythium mamillatum*	6
	Peas	*Aphanomyces euteiches*	7
	Allium spp.	*Sclerotium cepivorum*	8
	Tomato Radish Lettuce	*Fusarium* spp.	9
	Beans	*Fusarium solani* f. *phaseoli*	10
	Banana (var. Gros Michel)	*Fusarium oxysporum* f. *cubense*	11
Spore-germination inhibitors	Banana (var. Lacatan)	*Fusarium oxysporum* f. *cubense*	11
	Peas	*Fusarium oxysporum* f. *pisi*	12
Zoospore attractors	Strawberry	*Phytophthora fragariae*	13
	Peas	*Phytophthora erythroseptica*	14
	Solonaceae	*Phytophthora parasitica*	15
	Avocado	*Phytophthora cinnamomi*	16
Pathogenicity stimulator	Peas	*Fusarium oxysporum* f. *pisi*	17
Microsclerotia-germination stimulators	Tomato Wheat	*Verticillium albo-atrum*	18

References: [1] Kerr, 1956; [2] Husain and McKeen, 1962; [3] Schönbeck, 1958; [4] White, 1954; [5] Ebben and Williams, 1956; [6] Barton, 1957; [7] Scharen, 1960; [8] Coley-Smith, 1960; [9] Jackson, 1957, 1960; [10] Schroth and Snyder, 1961; [11] Buxton, 1962; [12] Buxton, 1957; [13] Goode, 1956; [14] Bywater and Hickman, 1959; [15] Dukes and Apple, 1961; [16] Zentmyer, 1961; [17] Buxton, 1958; [18] Schreiber and Green, 1962.

release of amino acids from roots in sand or soil. Although this situation of wilting does not arise in most laboratory exudate studies, it could be important in the field, where periods of temporary moisture stress often occur. The rapid release of amino acids from roots of wilted plants upon rewetting will exert a profound effect on the numbers and types of organisms in the rhizosphere.

Exudation is also affected by other environmental conditions. It is increased under high light and temperature conditions and is greatest during the first few weeks of growth (Rovira, 1959). There are no reports available on the influence of transpiration, but it would be surprising if this did not exert some effect. One of the criticisms of using the enclosed-tube technique for the aseptic culture of plants in exudate studies may be that transpiration is limited under these conditions.

Early in this review emphasis was placed on the necessity of growing plants under aseptic conditions in root-exudate studies. This is supported by the work of Martin (1958), who found that the culture filtrates of certain bacteria caused a threefold increase in the exudation of scopoletin without affecting root growth, whereas other culture filtrates had no effect on exudation. The amino acid patterns in exudates from tomato and clover roots were altered by the presence of microorganisms (Rovira, 1959), probably owing to the utilization of certain amino acids and release of others by the microorganisms. The reports of Ljunggren and Fåhraeus, discussed earlier, on polygalacturonase released from roots under the influence of compatible rhizobia are further evidence of the part microorganisms play in the behaviour of plant roots. The observation by Norman (1955) that polymyxin and other polypeptide antibiotics increased the leakage of inorganic and organic materials from roots indicates the effects colonies of antibiotic-producing microorganisms may have upon exudation.

Microorganisms can affect exudate patterns in at least three ways: by altering the permeability of root cells, by modifying the metabolism of roots, and by modifying some of the material released from the roots. In the rhizosphere, where there is an intense development of microorganisms, it would be surprising if exudation were not affected by the presence of organisms or their metabolic products. In this respect, the root should not be considered uniformly populated; it probably has zones of intense microbial colonization and other zones of lower population density. If, in these areas of intense colonization, there develop species of organisms releasing substances which increase the permeability of underlying root cells, then predictions of food supplies to the rhizosphere population on the basis of root-exudate studies carried out under aseptic conditions could be misleading.

In view of these effects of microorganisms on exudation, studies should be conducted under both sterile and nonsterile conditions to assess the potential and actual supply of nutrients in the rhizosphere. The rhizoplane and rhizosphere microflora may be regarded as a selective sieve, absorbing some exudate constituents and, in turn, releasing exudates from their own cells. By studying the materials in the environment around nonsterile roots, information is obtained of the situation existing when plants are growing in the field—this being the environment concerned in the biological control of root pathogens.

The results of Buxton (1960) support the hypothesis that caution is necessary when interpreting actual rhizosphere ecology in terms of the results obtained with root exudates produced under aseptic conditions. He showed that although exudates from peas susceptible to *Fusarium oxysporum* stimulated the fungus in pure culture, they also caused many rhizosphere isolates to antagonize the pathogen; thus there were in fact two opposing systems operating in the rhizosphere of susceptible pea plants. He suggested that "Under natural conditions root exudates are unlikely to be the dominant factors in the rhizosphere environment, for the microflora will rapidly change the chemical nature of the exudates and, in doing so, may acquire metabolic activities not normally detected in the rhizosphere." Although tested with only one host species, its pathogen, and a range of rhizosphere isolates, Buxton's findings and hypothesis could have a general application and should be considered in planning further studies of exudate-pathogen-saprophyte interactions.

MECHANISM OF EXUDATION.—The use of various terms to describe the release of material from plant roots—excretion, exudation, secretion, diffusion, and leakage—indicates that little is known of the mechanisms involved in this process.

Apart from the studies of Lundegårdh and Stenlid (1944), who linked exudation with respiration, no attempts have been made to study the problem at a physiological level. This is because plant physiologists are more interested in the movement of nutrients into plants than in the reverse phenomenon: All the published work other than that of Lundegårdh and Stenlid has been by microbiologists or plant pathologists, who were mainly concerned with exudation as a source of nutrients of microorganisms rather than with the mechanisms. More recently Rivière (1959) has proposed that exudation should be considered a manifestation of the permeability of cells at the particular stage of growth being studied and linked with the metabolism of root cells, but he produced no evidence to prove this statement.

Until more is known of the processes involved in the release of organic material from roots, it is probably best to use the terms exudation or diffusion to describe the process. According to definition these terms mean "oozing out, giving off, sending forth, or shedding abroad." The terms excretion and secretion, on the other hand, imply active physiological mechanisms, while "leakage" implies movement of fluid through perforations.

SIGNIFICANCE OF EXUDATES IN THE RHIZOSPHERE.—In spite of the extensive evidence for exudation of organic material from roots and the specificity of some of these materials in relation to certain soil microorganisms, there is little conclusive evidence of the relative importance of original exudate, exudate modified by microorganisms, and sloughed-off cell debris in the establishment and maintenance of the rhizosphere population during the life of the host plant.

Results with peas and oats indicate that exudation is more important than cell debris in the young seedling, but that as the plant develops exudation declines and decomposition of moribund root hairs, epidermal cells, and cortex contributes a substantial portion of the nutrients to the rhizosphere population (Rovira, 1956*a*). Root exudates no doubt will still be the major factor for some distance behind each growing tip of the root system, but they will contribute proportionately less as the plants develop.

My direct observations support the evidence of Rogers, Pearson, and Pierre (1942) and others that a considerable amount of cell material in the form of cast-off root-cap cells accumulates around the roots during growth. The quantity and viability of these cells will depend upon the plant species and the medium through which the roots are growing. Figures 2 and 3 show two stages in the decomposition of root hairs of *Phalaris tuberosa* grown in sand for 6 weeks. In Fig. 2 bacteria are seen inside what appears to be an intact root hair, probably at an early stage of decomposition. Fig. 3 shows three portions of a single root hair completely ensheathed by bacteria, indicating an advanced stage of decomposition.

DIRECT MICROSCOPY OF ROOT-SURFACE MICROFLORA.—Direct examination of roots stained with aniline blue (Rovira, 1956*c*) often shows distinct patterns of development of microorganisms over the surface of the root. With tomato roots (Fig. 4) bacteria tend to develop along the root on what appear to be the junctions of epidermal cells. On *Phalaris* roots (Fig. 5) the bacteria often develop as distinct colonies, ranging in size from a few cells to many hundreds of cells per colony. It is not known whether these patterns are manifestations of differences in sites of exudation between the two plants or due to differences in the surface structures of the respective roots.

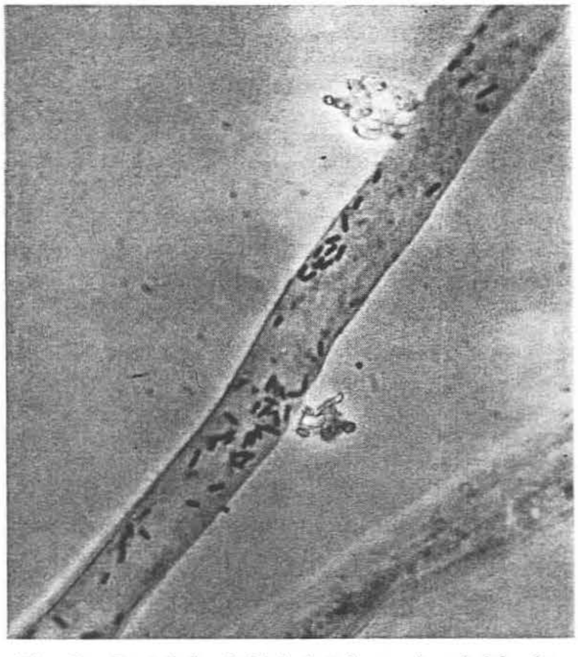


Fig. 2. Root hair of *Phalaris tuberosa* invaded by bacteria. (× 1,000)

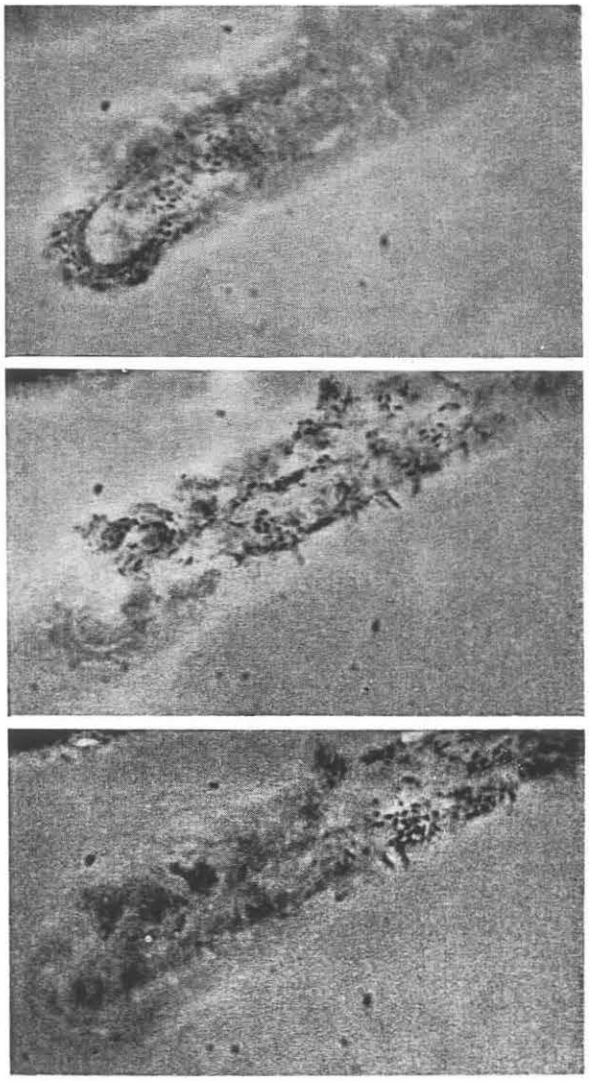
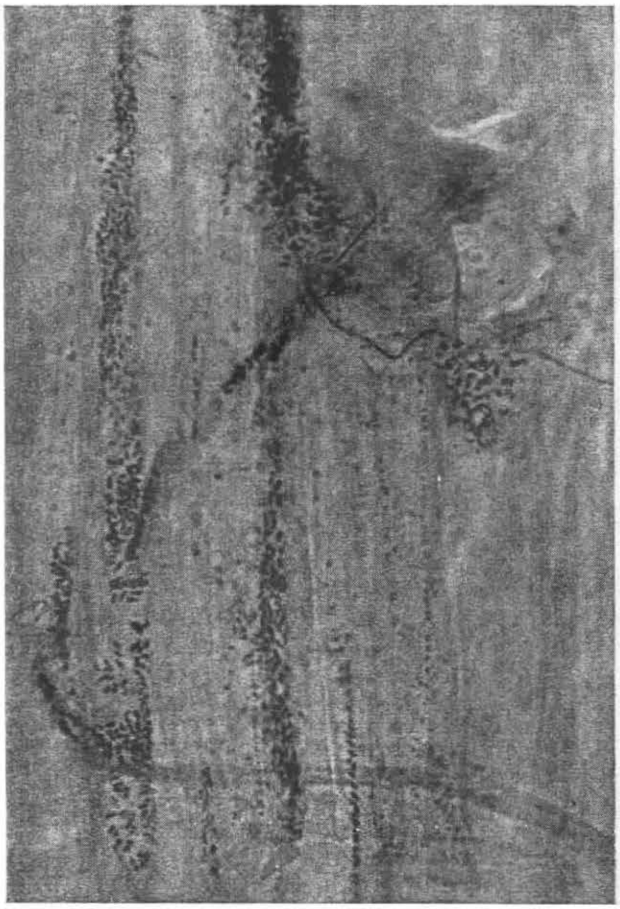


Fig. 3. Moribund root hair of *Phalaris tuberosa* colonized by bacteria. (× 1,000) Three portions of roots shown by focusing at different levels.

Although direct observations by the above methods are useful to indicate the distribution of the microflora on the root surface, little information can be obtained on the types and identity of these organisms. By using the fluorescent-antibody technique, I have been able to stain specific groups of bacteria in situ and obtain information on the distribution of serologically different organisms on the root surface. Further development of this technique should yield valuable information on the colonization of roots by different organisms, the relative proportions of different types, and the degree of com-



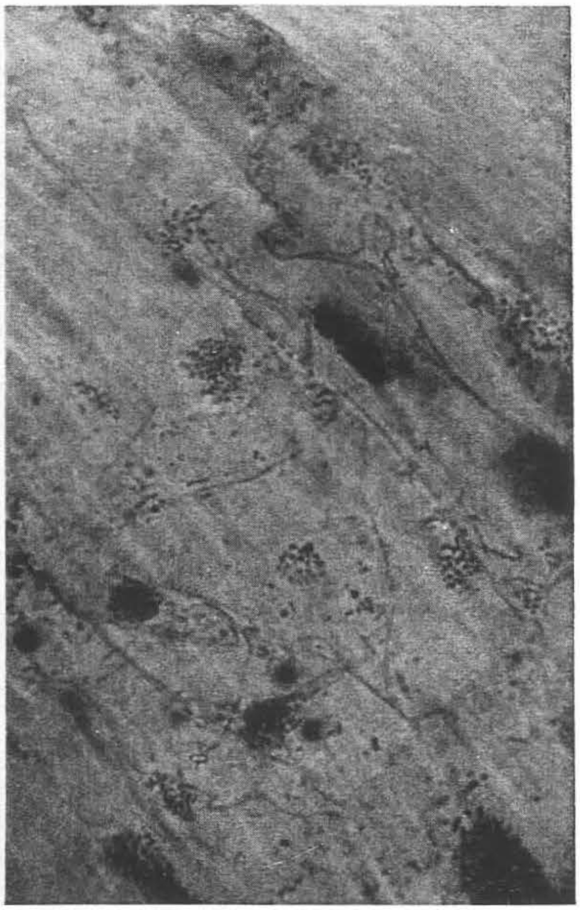


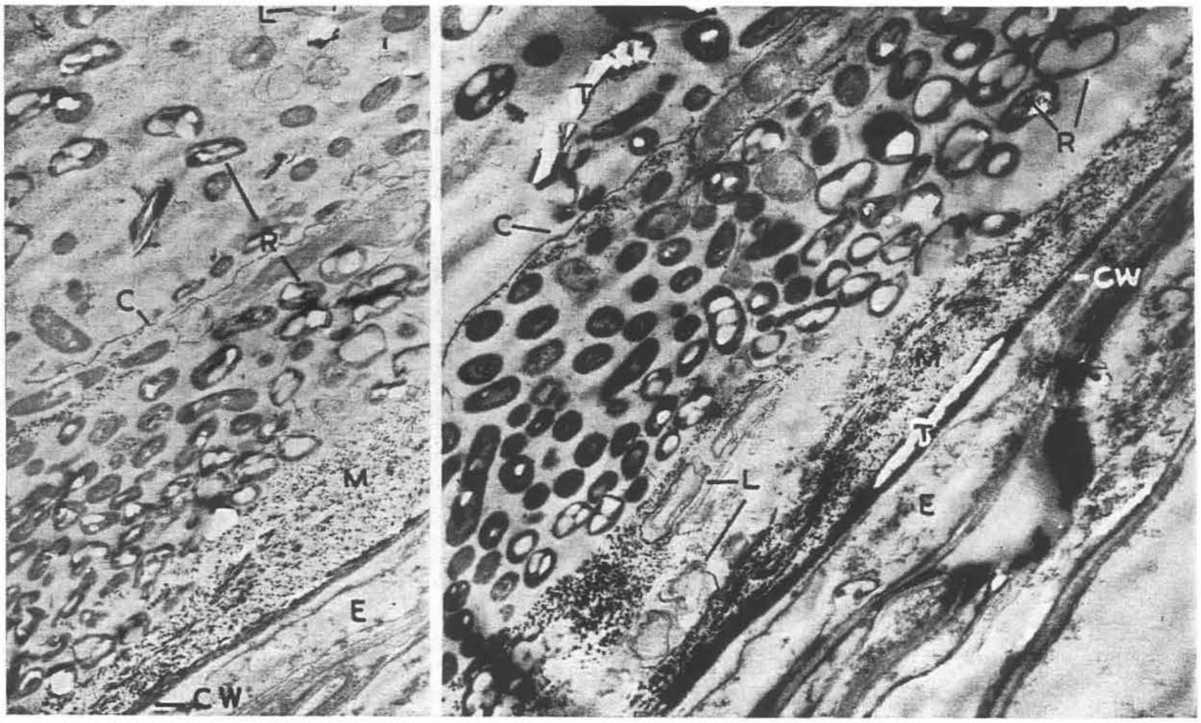
Fig. 4 (left). Development of microorganisms on roots of tomato. (× 1,000)
Fig. 5 (right). Development of microorganisms on roots of *Phalaris*. (× 1,000)

petition of root-surface organisms for nutrients from root exudates. That this technique can be used successfully for fungi as well as bacteria has been demonstrated in medical microbiology (Kaufman and Kaplan, 1961) and in the detection of *Aspergillus flavus* in soil by Schmidt and Bankole (1962). The principles and technical details involved in this fluorescent antibody method have been reviewed by Beutner (1961).

Electron microscopy has been used successfully by Dart and Mercer (1964) to study the colonization of the roots of barrel medic (*Medicago tribuloides*) by *Rhizobium meliloti*. There was an intense colonization of the mucilaginous sheath (Fig. 1) by bacteria within two days of inoculation with an effective strain of *R. meliloti* (Figs. 6 and 7). The bacteria colonized between the mucilaginous material and the "cuticle," forcing the latter away from the roots. Some cells are visible within the mucilage but most of these appear to have undergone lysis.

THE USE OF "MODEL SYSTEMS" IN ROOT-EXUDATE STUDIES.—The complexity of the rhizosphere environment in natural soil makes it difficult to resolve such questions as the interactions between organisms, the numbers of viable organisms, and the characteristics required for an organism to become an inhabitant of the rhizosphere. For these reasons "model" systems which simplify the situation have been developed.

Chan and Katznelson (1961, 1962) have developed "model systems" where cultures, single or mixed, are grown in media with and without root extracts. In root-extract-supplemented medium, they have noted patterns of development in the mixed cultures very similar to those found around plant roots. When cultures of five bacteria were grown together in the presence of oat and soybean root extract, *Azotobacter chroococcum* died, *Bacillus cereus* was a poor competitor, while *Pseudomonas* sp., *Agrobacterium radiobacter*, and *Arthrobacter citreus* all grew well, with *Pseudomonas* giving the highest numbers of cells. This type of experiment with different organisms and concentrations of root exudate could lead to a better understanding of the mechanisms of competition, stimulation, and antagonism which occur in the rhizosphere. The work of Chan and Katznelson has been performed with root extracts rather than root exudates, although there are no data available comparing the contents of these materials. Some information on the behaviour of the five cultures in both root-exudate and root-extract-supplemented media



Figs. 6 and 7. Development of ***Rhizobium meliloti*** beneath the "cuticle" of the mucilage on the root of *Medicago tribuloides* 5 to 15 mm from the tip. (× 8,700) *E,* epidermal cell; *C.W.,* epidermal cell wall; *M,* mucilaginous layer; *C,* "cuticle"; *R, Rhizobium* cells; *L,* possibly lysed rhizobia; *T,* section tear. (P. J. Dart and F. V. Mercer. Arch. Mikrobiol. 47: 344-378. 1964.)

would indicate the value of root extract (which is simpler to prepare) as a substitute for root exudate.

Contrary to the findings of Chan and Katznelson, Meshkov and Khodokova (1954) and Zinov'eva (1958) found that the exudates of peas, maize, oats, and wheat stimulated *Azotobacter chroococcum* more than other soil bacteria, including *Rhizobium* and Gram-positive organisms. These results were obtained with strains of *Azotobacter* "adapted" to the rhizosphere by 7 to 10 passages on the roots of the particular host plant. Possibly the use of adapted strains accounts for the discrepancies between the results of various workers.

Vancura and Macura (1961), in a study of the effects of root exudates on *Azotobacter chroococcum,* also found the exudates of wheat and barley stimulatory provided the organism had previously been "adapted" to growth on roots (Vancura et al., 1959). The organic-acid fraction of root exudate was the most stimulatory fraction to both growth and nitrogen fixation of *Azotobacter.* Of the sugar fraction, galactose and fructose were preferentially utilized by the organisms. In stationary cultures the amino-acid fraction of root exudate was inhibitory, but this was overcome with shaking.

A further extension of the idea of "model systems" is to inoculate seeds with cultures singly or mixed, plant in sterile sand or nutrient solution, and follow the development of the organisms as the plants grow. This method has the advantage that exudate concentrations may be more realistic than when exudates or extracts are added to nutrient media. Also, there is the physical environment of the root available if surface adhesion, water movement, ion exchange, or carbon dioxide and oxygen levels play significant roles in the rhizosphere phenomenon. With this method, *Azotobacter chroococcum,* even in pure culture, failed to colonize the roots of maize growing for 2 weeks in sterile sand, while *Bacillus polymyxa* and *Clostridium pasteurianum* reached 4 and 8 million per root system, respectively (Rovira, 1963). *Pseudomonas fluorescens* proliferated on the roots and reached 800 million per root system. With tomato, *Azotobacter* reached 40,000 cells per root system, but this was still only 1 per cent of the level reached by *Pseudomonas.*

An alternative technique in the more complex soil environment showed that an "artificial rhizosphere" may be created by continual additions of root exudates to soil (Timonin, 1941; Rovira, 1956*b*; and Rivière, 1958). The organisms isolated from soil treated in this manner were similar to those in the rhizospheres of the same plants; and although such a system lacks the nutrient depletion, water movement, and surface nature of the root, it could be useful in studying the effects of different root exudates or fractions thereof upon the complex array of microorganisms in natural soil. While it is admitted that there is a considerable difference between the environments of the model systems and the natural situation and that caution is required in reaching any conclusions, it appears that use of model systems of increasing complexity in root-exudate and rhizosphere studies will be necessary to understand the dynamics of the rhizosphere phenomenon.

Conclusions.—Sufficient chromatographic analyses have been performed on root exudates to indicate the wide spectrum of compounds they contain. More emphasis, however, should be placed on the effects of these exudates, complete and fractionated, on members of the rhizosphere microflora, both pathogens and nonpathogens. In such studies attempts should be made to keep the root-exudate concentration comparable to that which occurs in the rhizosphere. The question of concentration is very difficult to resolve because little is known of the volume of soil around the root into which the exudates diffuse and the extent to which the various constituents are adsorbed by the soil colloids. Possibly the solution lies in using a range of concentrations in the presence or absence of adsorbing materials. Further development of the principle of "model systems" involving plants and mixtures of known microorganisms offers considerable promise in explaining some of the mechanisms operating in the rhizosphere.

Research is also necessary on the effects of various factors such as environmental conditions and microorganisms (pure and mixed cultures) on exudation. A greater understanding of the mechanisms involved in exudation is also required. With more such information it should be possible to predict the behaviour of the rhizosphere microflora of a particular plant species under different soil and climatic conditions.

An interesting development in root-exudate research in relation to controlling the rhizosphere population via the exudate has been proposed by Vrany, Vancura, and Macura (1962). They applied a variety of substances—readily metabolized substances such as urea and inorganic phosphate, growth regulators, and antibiotics—to the foliage of wheat and found that they could modify root exudates and the rhizosphere microflora by such treatments.

The site of exudation in relation to the areas of development of the rhizosphere flora and root pathogens is an important area requiring further investigation. Pearson and Parkinson (1961) and Schroth and Snyder (1961) demonstrated that the root tip is the most important zone of exudation, whereas Frenzel (1960), by using the growth of specific nutrient-requiring mutants of *Neurospora,* found that threonine and asparagine came from the root tip, but leucine, valine, glutamic acid, and phenylalanine were exuded in greater amounts from the root-hair zone. These results indicate the possibility of different organisms developing in different regions of the root—a point which must be clarified if the biological control of root diseases is to be achieved through the rhizosphere population. The use of organisms themselves as indicators of exudation, together with direct microscopic examination under both phase and ultraviolet illumination, should provide useful tools to study this aspect.

In conclusion, it can be said that there is little doubt that root exudates play an important role in the establishment and maintenance of the rhizosphere populations of young plants, often being quite specific in their action. But considerable research is still required along the lines suggested above to assess their true significance in controlling the balance and numbers of organisms in the rhizosphere during the whole life of the plant. Further understanding of the dynamic situation operating in the rhizosphere resulting from root exudate studies will lead to the establishment of basic principles to be used in the biological control of plant root pathogens.

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► DISCUSSION OF A. D. ROVIRA PAPER

G. H. Hepting:

Do you feel that exudates from foliage and other aerial parts of plants would be likely to influence soil microflora?

A. D. Rovira:

Yes, there is evidence that significant quantities of organic materials are released from leaves and will be washed down into the soil. The amounts and types of materials coming from leaves varies according to plant species, age of plant, and climatic conditions.

There is certainly sufficient nutrient material in leaf exudate to affect the microflora in upper layers of the soil beneath the plant.

L. F. Johnson:

I would like to comment on Dr. Hepting's question about exudation from leaves. At the University of Tennessee we have found that certain plants have a distinct microflora on their leaves. *Streptococcus faecalis* is commonly spread from plant to plant by insects and can be found on the leaves and flowers of many plants. This association is specific, in that when seeds of certain plants are inoculated with this bacterium, it will grow along with the plant and become epiphytic on the leaves. It will not become epiphytic on other species of plants. Therefore, there is an apparent microflora associated with leaves. It is believed that this association is influenced by exudation from leaves. Dr. Rovira, how do you separate or distinguish between root exudates and leaf exudates that might be produced in your test-tube cultures?

A. D. Rovira:

The only precaution one can take when using the test-tube technique is to prevent any wetting of the leaves when changing the nutrient solution.

A. Burges:

1. Referring to Fig. 4: are the rows of bacteria in the longitudinal furrows, and if so, have bacteria which might have been on the ridges been washed off?

2. Regarding the possibility of using bacterial distribution as a means of locating leakage: while this could be used on the macroscale, would not diffusion invalidate the method on the microscale?

A. D. Rovira:

1. In the material used for the illustration, care was taken to avoid washing microorganisms from the roots so we can be confident that the linear orientation on tomato and colony development on *Phalaris* were the natural arrangements at the time of sampling. Briefly, the method consists of carefully removing sand or soil, and staining in Jones and Mollison aniline blue-phenol-acetic acid stain for 3 minutes.

2. Frenzel's experiments using mutants of *Neurospora* with different nutritional requirements show that the development of microorganisms on the root surface can be used to indicate sites of exudation, but there is no doubt that diffusion away from exudation points is a problem to be considered with this technique.

D. Parkinson:

You have stated that studies of root exudates should be made both on plants growing in sterile and in nonsterile culture; however, plant roots in nonsterile soil are surrounded by large populations of microorganisms which are probably more "leaky" than the plant roots. Surely, analyses of such nonsterile systems, from the viewpoint of exuded material, will be extremely difficult to interpret.

A. D. Rovira:

I agree with you, but I consider studies under nonsterile conditions an extension of detailed studies of exudation from roots growing under aseptic conditions.

F. M. Scott:

Grass root epidermis consists of long cells and short piliferous cells. Do bacterial colonies aggregate more densely on short cells?

A. D. Rovira:

I cannot say because I did not observe very closely the patterns of epidermal cells beneath the rhizoplane microflora.

W. D. Thomas:

How prevalent are such acids as chlorogenic and tannic acids in root exudates? Is it likely that all chemical constituents of root cells may show up in tree root exudation?

A. D. Rovira:

To my knowledge there are no analyses for these acids in root exudate. Amino acid analyses do indicate a similarity between the internal and external amino acids but such analyses of extracts of entire root systems may differ from the amino acids in the cells at the sites of exudation.

S. Wilhelm:

Roots of plants growing in nature or under cultivation are commonly and abundantly infected by parasitic fungi, especially those belonging to the genera *Olpidium* and *Endogene* (*Rhizophagus*). Root secretions in nature may thus be largely from parasitized roots and the spectrum of chemicals far greater than envisioned from roots growing under aseptic conditions.

The idea is old that plant roots may secrete complex chemicals involved in stimulation of other organisms. P. A. Mitcheli (Relazione dell' erba detta da' botanici *Orobanche;* Firenze, 1723) suggested it in his discussion of control of *Orobanche* on *Vicia faba.* J. P. Vaucher (Mém. Mus. Hist. Nat. 10: 261-273; 1823) considered it in his detailed studies on germination of seed of *O. ramosa.* R. Wight (Spicilegium Neilgherrense, vol. 2; Madras, 1851), a British physician stationed in India, considered root secretions of tobacco so obviously involved in the question of *Orobanche* seed germination that experimental demonstrations of them were unnecessary. R. M. Barcinsky (Dokl. Akad. Nauk SSSR (N.S.) 1: 343-346, 1934; *ibid.* (N.S.) 2: 311-313, 1935) and B. S. Zakharov (Comptes Rend. [Dokl.] Akad. Nauk. URSS 27: 267-270, 1940) showed that day length may profoundly influence the susceptibility of sunflower to *Orobanche* and implicated root secretions as bringing about the effect.

D. Pramer:

Will you comment on the possibility of controlling soil-borne plant pathogens by treating foliage with compounds that are absorbed by leaves, translocated downward, and exuded by roots?

A. D. Rovira:

Some excellent work on this topic has been done by Vrany, Vancura, and Macura (cited in the paper) in Czechoslovakia, who showed that the application of chloramphenicol to the leaves of wheat not only led to this antibiotic being exuded but also markedly increased the exudation of amino acids and sugars.

R. A. Kilpatrick:

Does the lack of aeration of sterile plants grown in agar affect the rate of release of root exudates?

A. D. Rovira:

Possibly; very little is known about this but the diffusion of oxygen through soft agar differs very little from diffusion through water.

R. A. Kilpatrick:

At the University of New Hampshire, red and white clovers have been grown under sterile conditions (test tubes) under about 400 ft-c fluorescent light. For 4 months, plants appear to grow normally; soon after this period, plants begin to die. Dead plants were removed and the agar was diluted with water, filtered, and then run through several chemical tests. Finally, a white substance was obtained. This was dissolved in water, sterilized, and then used to test the effect on seed germination of white clover. Nontreated seeds germinated 100%. The exudate suspension completely inhibited seed germination. Identification of the compound is unknown at this time.

H. Burström:

Different modes of exudation known from other plant parts will give different types of compounds, and it might thus be of interest to know which are operating in roots. Passive leakage through free space will give any permeable, small-molecular compounds. Active squeezing out of vacuolar sap, occurring in hairs and glands, will give everything stored in the vacuole. This requires pits or ectodesmata. Active exudation from the cytoplasm by the mechanism of phloem and nectaries, according to Lüttge, will give cytoplasmic compounds: sugars, amino acids. Emptying of dying cells will give the whole cell content.

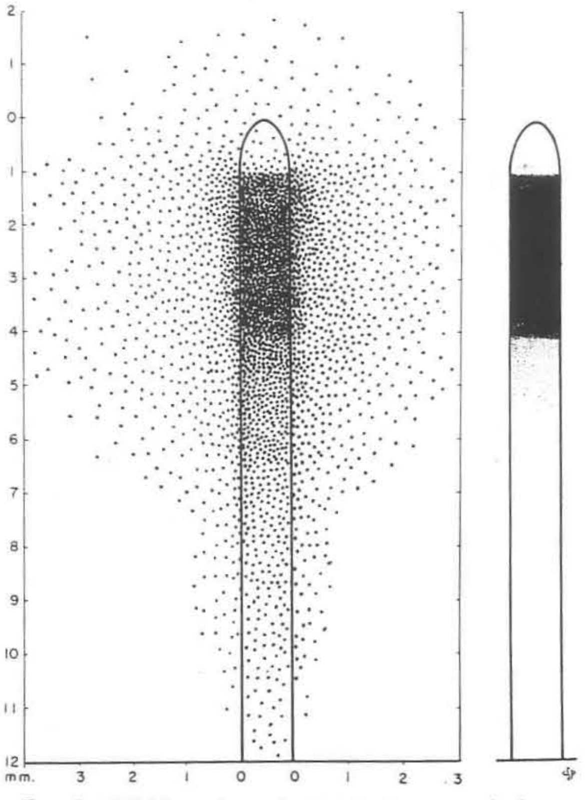

Fig. 8. Within a few minutes of placing excised avocado roottips in a suspension of actively swimming zoospores of *Phytophthora cinnamomi,* the spores are attracted to the root, and particularly to the region of elongation, as indicated in the diagram. In 24 hours the initial root lesion (right) develops at this area of abundant spore attraction and encystment. (See text, p. 186.)

A. D. Rovira:

Thank you, Dr. Burström. I hope that we can encourage plant physiologists to study these mechanisms which you suggest because an understanding of them is essential in any study of the rhizosphere phenomenon.

G. A. Zentmyer:

Zoospores of *Phytophthora cinnamomi* exhibit striking chemotaxy to the region of elongation on avocado roots, which constitutes further indirect evidence of the increased exudation of materials from this area of elongation, as compared with other areas of the root (Fig. 8).

G. Stotzky:

Although we all appreciate the difficulties involved in growing plants with axenic roots, as well as the necessity for growing large numbers of plants, I wonder if oversimplication of the culture apparatus doesn't sometimes defeat the purpose of the experiment. For example, most studies have been restricted to investigating the exudates from young seedlings, and little effort has been made to separate the root zone from the seed. Consequently, is it not possible that a sizable portion of the organic compounds isolated from the culture solution of these seedlings are breakdown products of the endosperm rather than "true" root exudates? The contribution of materials from the endosperm would be especially large with plants like peas, and this source may account for the large quantities of reducing sugars reported to be present, 2 weeks after germination, in culture solutions supporting peas.

A. D. Rovira:

This is a good point, but we consider that by pregerminating the seed on agar before planting in our tubes, we dispose of the bulk of the seed exudate. By having the seeds on stainless steel screen 1 cm above the solution, we consider that most of the material obtained must come from the roots. The fact that we find no differences between the exudates of roots developing from different-sized seeds of one plant species supports our contention that we are analysing root exudates. Further evidence comes from the fact that exudates vary according to the light intensities under which plants are grown. We would not expect light intensity to affect seed exudation.

R. D. Durbin:

Another factor which may influence root exudates is the presence of a disease in the aboveground portion. We have found that rust-infected bean leaves translocate only ½ to 1/10 the quantity of photosynthates to the root system that healthy plants translocate, and soon after infection the rate of root growth declines. Last in England has found that powdery mildew begins to adversely affect root growth of barley several days after inoculation. Quantitative and perhaps qualitative changes in root exudates possibly could result from this and may in part be responsible for predisposing the plant to root-invading microorganisms.